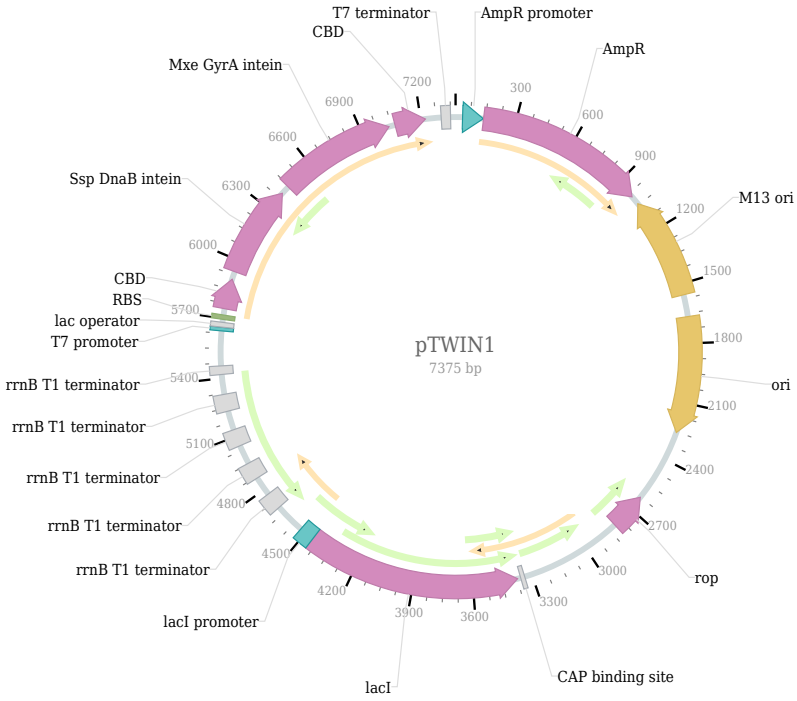
pTWIN1

载体编号 Vector ID	V012684
抗性 Antibiotic Resistance	Ampicillin
长度 Length	7375 bp
载体类型 Vector Type	Bacterial expression vector

产品信息

复制子 Replication Origin	ori
来源 Source/Author	NEB
拷贝数 Copy Number	High copy number
启动子 Promoter	T7
克隆方法 Cloning Method	Enzyme Cut
融合标签 Fusion Tag	intein
表达方式 Expression Method	IPTG induced
备注 Comment	pTWIN1 is an E. coli plasmid cloning vector designed for recombinant protein expression, labeling, and cyclization using the IMPACT-TWIN Kit (NEB #E6901) (1). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTWIN1 also contains an M13 origin of replication. The multiple cloning site (MCS) is positioned to allow translational fusion of an intein tag to the N-terminus, C-terminus, or both, of the cloned target protein. The mini-inteins encoded by pTWIN1, the Ssp DnaB intein and the Mxe GyrA intein, cleave the peptide bond at their C- and N-termini, respectively (1-4). The chitin binding domain (CBD) from B. circulans, fused to each intein, facilitates purification of the intein-target protein precursor. Transcription of the intein tags is controlled by the inducible T7 promoter, requiring E. coli strains containing integrated copies of the T7 RNA polymerase gene [e.g., NEB #C2566, #C2833 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the lacI gene, to the lac operator immediately downstream of the T7 promoter (5). Translation of the intein tags utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

质粒图谱



复溶方法

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.